Integrationvectors for producing genes, which produce recombinant antibodies.

Publication number: EP0675203
Publication date: 1995-10-04
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Classification:
- International: ***C07K16/00;*** **C07K16/00;** (IPC1-7): C12N15/63
- European: C07K16/00
Application number: EP19950102032 19950214
Priority number(s): DE19944406512 19940228; DE19944419254 19940601

Also published as:
EP0675203 (B1)

Abstract of **EP0675203**

Integration vectors for the prodn. of genes encoding recombinant antibodies are claimed. They comprise: (a) a region of >= 1.5 kb homologous to a region of the mu - or k-intron not including a C mu or Ck enhancer;(b) >= 1 DNA sequence encoding >= 1 domain of an antibody, or(c) a marker, selectable in eukaryotic cells, producing antibodies which lack a functional enhancer region, the expression of this marker being controlled after the integration of cellular C mu - or Ck enhancer. Also claimed are:(1) a method of producing recombinant antibodies by:(i) using the vector above;(ii) selecting stable transformants, and(iii) detecting cells producing the desired antibody, and(2) a kit for the prodn. of antibodies contg. the new vector.

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The invention concerns integration vectors for the production of genes, which code rekombinante anti-bodies, procedures for the production of rekombinanten anti-bodies using these vectors, procedures for the production of Zellinien using these vectors, which produce only the rekombinanten anti-bodies, as well as kits for the production of rekombinanten anti-bodies, which contain at least one of these vectors.

Homologous recombination between exogenous and chromosomalen DNA sequences experienced applications within many ranges of modern biology. Thus over homologous recombinations the Genom of embryonaler main cells and further mammal cell types was changed specifically (see. e.g. Capecchi, Science 244 (1989), 1288-1292). For example the gene function was destroyed by these changes, mutations were corrected or slight changes in the nucleotide sequence of a gene was made (see. e.g. Davis et al., mol. Cell. Biol. 12 (1992), 2769-2776). One knows that exogenous nucleic acids are built sequence-specifically into yeast (Orr Weaver and Szostak, Microbiol. Rev. 49 (1985), 33-58). In mammalian cells the installation takes place however with small frequency by means of homologous recombination in favor of a substantially more frequent coincidental integration.

On the basis of this realization was tried to develop effective selection and searching procedure which make the isolation possible of mammalian cells with a desired recombination event. Mansour for example in addition a positive-negative selection procedure suggested (Mansour et al., Nature 336 (1988), 348-352). One knows meanwhile the fact that the efficiency of the homologous recombination of the length of the homologous sequences depends (see. e.g. Berinstein et al., mol. Cell. Biol. 12 (1992), 360-367), as is affected also by the Transkriptionsaktivität of the goal sequence (see. e.g. NIC getting off, mol. Cell. Biol. 12 (1992), 5311-5318)

For homologous recombination experiments two different in principle vectors are available, which are called ?integration? and ? Replacementvektoren?. Integration vectors contain a homologous flanking sequence, into which a break of the DNA Doppelstranges is inserted. With the recombination event the entire vector is integrated into the Genom, which leads to a Duplikation of a part of the goal sequence. Replacementvektoren against it contain a homologous sequence, into which a heterologer range is inserted (for example a gene for a selection marker). This kind of vector is linearized before the Transfektion at the end or outside of the homologous region. The integration sample of integration vectors in yeast and mammalian cells can be predicted by the doubling rank break repair model developed by Orr Weaver and Szostak sufficiently (Orr Weaver and Szostak, a.a.O., Szostak et al., Cell. 33 (1983), 25-35). Replacementvektoren with not-homologous ends seem to use however another integration mechanism, since their free strand ends seem to play a less important role for the kind of integration than from integration vectors (Hasty et al., mol. Cell. Biol. 12 (1992), 2464-2474)

Is of special importance for a high number of homologous recombination events the use of isogener DNA (see. e.g. Deng and Capecchi, mol. Cell. Biol. 12 (1992), 3365-3371), since cousin wrong mating lowers the recombination frequency within the homologous region drastically. If ISO genes homologous sequences are used, integration and Replacementvektoren are similarly effective. However if non-ISO genes sequences are used, then with Replacementvektoren less wished recombination events are obtained. This is to be attributed to the fact that the integration of Replacementvektoren requires two recombination events. With cousin false mating it comes by it less frequently to a successful homologous recombination than with integration vectors, only one recombination event needs (Deng and Capecchi, a.a.O.).

The homologous recombination technology one made oneself also for the construction of chimärer anti-bodies. A multiplicity of monoclonalen mouse anti-bodies with therapeutically interesting specificities are worthless for the therapy in humans, since its application causes the induction of anti-body directed against it. This leads to a neutralization of the anti-bodies and thus the therapeutic effect. Besides can unwanted side effects will cause. Skin et al., Proc. Natl. Acad. Sci. The USA 86 (1989), 8507-8511 linked the variable region of the heavy chain of a mouse anti-body over homologous recombination using an integration vector with human C gamma 1-Region. They used thereby a Konstrukt which contained the mouse JH4-Region, a part of C mu - Introns with C mu - of Enhancer, connected with human C gamma 1-Exons as well as a selection marker. With this Konstrukt have skin et al. a frequency from a functional genomischen recombination in 200 Transfektanten receive. A such frequency at homologous recombination events makes a relatively complex searching procedure still necessary after the desired clones.

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On the other hand human Zellinien is available, which sezernieren anti-bodies of certain specificity. Would be desirable also here, if these anti-bodies will provide in a simple manner with other specificities, which originate for example from the mouse or can synthetic origin be, could. So far such rekombinanten anti-bodies were essentially manufactured by the rekombinante DNA technology in non-producing Zellinien with very small yield. This is a complex procedure, which is unsuitable for the rapid change of anti-body specificities.

The available invention was the basis therefore the technical problem to make vectors available with which a large frequency of homologous recombinations in Immunglobulinloci can be obtained, whereby with larger frequency than with while stationary the technology vectors functional rekombinante anti-bodies admitted that be made available can. The solution of this technical problem is obtained by the execution forms represented in the requirements.

Thus the invention concerns an integration vector for the production of genes, which code rekombinante anti-body, which contains the following elements:

(A) a range from at least 1.5 KB, which homologous to a range mu - is Introns or k-Introns, which no or no functional C mu - or CC-Enhancer contains;
(B) at least one DNA sequence, which codes a domain of an anti-body or a part of it; and
(C) a marker selectable in eukaryontischen anti-body-producing cells, which does not exhibit a functional Enhancer range, whereby the Expression of this marker is steered after the integration by cellular C mu - or CC-Enhancer.

The vector according to invention can exhibit one to a Intron richly homologous range from at least 1.5 KB, in which the industrial union-Enhancer does not natural-prove occurs or from which it was deletiert or in which it was inactivated.

The DNA sequence can cover for example one or more Exons, as long as a functional domain of an anti-body or a functional part of it is

coded. If the functional part of the domain is part of a V-domain, then this must be capable of the connection to the goal antigen or contribute to it. If it concerns a part of a C-domain, then this must be able to exercise at least one part of the Effektorfunktionen.

In a preferential execution form the range covers mu - or k-Introns of at least 1.5 KB the range, within which C mu - or CC-Enhancer is located, whereby this range no functional C mu - or CC-Enhancer more contains.

The Enhancer can be in this execution form either deletiert or inactivated. Such inactivating can e.g. procedures admitted to be caused by Mutagenese after while stationary the technology.

With in eukaryontischen anti-body-producing cells selectable marker the Enhancer was preferably deletiert or inactivated. In another execution form the marker does not exhibit natural Enhancer.

With the integration vector according to invention favourable-prove different V-genes or different ISO types leave themselves into a functionally rearrangiertes anti-body gene to in-recombine. The integration vector must exhibit a sequence homologous to the genomischen sequence contrary to an appropriate Replacementvektor only. Thus none must the respective endogenous constant region homologous sequence, like with that while stationary the technology admitted Replacementvektoren, into the vector to be built. If beyond that the used Hybridom is non-ISO towards to the vector sequence, a higher yield can be obtained than i.e. with a Replacementvektor, with the integration vector. the same integration vector can be applied to Hybridome of different mouse trunks.

The homologous sequence contained in the vector must exhibit a length of at least 1.5 KB, in order to be able to obtain a homologous recombination event at all. This DNA sequence of at least 1.5 KB can be selected from different ranges of C mu - or CC-Introns. The Enhancer is not according to invention in the Konstrukt contained or from of the Homologieflanke deletiert and/or. inactivated therein. During the integration of the vector into the homologous sequence of a functionally rearrangierten anti-body gene the Expression of the rekombinanten gene is placed under control of the endogenous Enhancers. Become if Exons, which code constant regions, in-recombines, then these stand furthermore under control of the endogenous V-activator. The Enhancer the Expression selectable Markers.Dadurch is guaranteed adjusted beyond that that the selection marker becomes active only if it is integrated in the proximity of a strong Enhancers. A homologous recombination with the Immunglobulinlocus is favoured by the used Homologieflanke, whereby the selectable marker is placed under control of endogenous C mu - or CC-Enhancers.

The term ?rekombinanter anti-body?, how uses here, designates everyone does not natural-prove occurring anti-body. The DNA sequences of natural, synthetic or semi synthetic origin inserted in the integration vector can be. Like natural anti-bodies coding sequences to be kloniert and/or. as synthetic or semisynthetische DNA sequences are manufactured, is well-known the specialist from the state of the art; see. e.g. Sambrook et al., ?Molecular Cloning, A Laboratory manual? 2. Edition 1989, Cold jump Harbor Laboratory, Cold jump Harbor, the USA, as well as Harlow and Lane ?Antibodies, A Laboratory manual? 1988, Cold jump Harbor Laboratory, Cold jump Harbor, the USA.

In a preferential execution form of the integration vector according to invention the DNA sequence codes a V-domain or a part of it. Preferably the V-domain is coding sequence a Vk-gene, if the integration event in an endogenous k-gene is to take place. If the recombination event is to take place into heavy chain gene, the V-domain is preferably coding sequence a front spar gene. The V-region can exhibit each possible antigen specificity. In this execution form the selection marker 5 is arranged ' of the sequence coding the V-domain, while the Intron range 3 ' is positioned of it. If the DNA sequence codes only one part of a V-domain, then this must be suitable for antigen connection.

In another preferential execution form the Inteörationsvektor exhibits a DNA sequence, which codes a constant region or a part of it. This sequence can code either for the heavy or the light chain of an anti-body. The specialist it is well-known that with all ISO types several Exons for the heavy chain code. If only a CH-Exon for the heavy chain is present in the Konstrukt, this is preferably the CH1-Exon. With a such Konstrukt rekombinante anti-bodies can be manufactured, which exhibit the functionality of Fab or F (off) 2-Fragmen-ten. Preferably the integration vector according to invention contains however all Exons heavy of a type of chain ISO, whereby a complete heavy chain can be exprimiert. In this execution form the elements (A), (B) and (C) in this order are successive in 5 ' - > 3 ' direction arranged.

In another preferential execution form of the integration vector according to invention exhibits the range homologous to mu - or k-Intron a length of at least 1.9 KB.

In a further preferential execution form of the integration vector according to invention this exhibits k-Intron homologous range a length of at least 2.0 KB to mu - or.

In a further preferential execution form the integration vector according to invention contains a bacteriacompatible regularization unit. A such bacteriacompatible regularization unit makes the Klonierung and Amplifizierung possible of the vector in bacterial systems, for example in E. coli. Bacteriacompatible regularization systems are well-known the specialist from the state of the art; see. Sambrook et al., a.a.O. An example of a such bacteriacompatible regularization unit is the regularization unit from the Plasmid pBR322.

In another preferential execution form the integration vector serves for the production of a rekombinanten anti-body, which is a chimärer anti-body. Under ?chimärer anti-body? an anti-body is understood here, which combines the v and C-regions from different species. For example a V-gene from the mouse with the C-Exons of a human type of ISO can be combined.

In another preferential execution form of the vector according to invention the DNA sequence codes domains of a human anti-body chain. These domains can be both V-as also C-domains or parts of it.

In a further preferential execution form of the vector according to invention the DNA sequence codes domains, which originate from the mouse, rat, goat, from the horse or sheep. Preferably all DNA sequences for either the v or the C-regions originate from one of these animal species. The invention covers however also such execution forms, in which the sequences coding the C-regions were inferred from different animal species.

As also according to invention the human domains coding DNA sequences used in another execution form of the vector these domains coding DNA sequences can be used for homologous recombination with the appropriate sequence from another kind of mammal.

In another preferential execution form of the vector according to invention the DNA sequence codes all C-domains of a sekretorischen anti-body.

In a further preferential execution form of the integration vector according to invention the DNA sequence codes all C-domains of a diaphragm-bound anti-body.

A further preferential execution form of the vector according to invention contains a DNA sequence, which codes C-domains of an anti-body, which is a IgM, a IgG1, a IgG2a, a IgG2b, a IgG3, a IgG4, a IgA, a IgD or a IgE Antikörper. The specialist it is well-known that some of these ISO types do not occur in all mammal species. Thus the human Genom contains for example of C-genes, which code IgG4-Isotypen, not however IgG2a-oder IgG2b-Isotypen. On the other hand the mouse Genom contains C-genes, which code the IgG2a and the IgG2b-Isotyp, not however the IgG4-Isotyp.

In a further execution form of the vector according to invention the rekombinanten anti-body-producing cells are human cells or mouse cells or hybrids of it. Mouse cells are preferred if the integration vector contains a human C-gene/human C-genes, which is to be in-recombined/downstream by the V-region of a rearrangierten mouse anti-body gene (EN). In such a way exprimierte anti-body can be preferably used for example for diagnostic and for therapeutic procedures. Further for therapeutic procedures anti-bodies which can be used are preferably manufactured in human cells.

So can e.g. V-genes from the mouse with desired specificities into a functional human Immunglobulin gene, a IgG1 or a IgG3-Gen to be preferably in-recombined. Furthermore the use of human cells has the advantage that the anti-bodies will provide with a human Glykosilierungsmuster, which does not cause unwanted side effects with therapeutic application.

The specialist is well-known, as it can make hybrids of human cells and mouse cells. Such hybrid can be manufactured for example by fusion of mouse B Lymphoblasten with human Myelomzellen.

Into all these cells synthetic V-genes can be in-recombined with the integration vector according to invention furthermore. If the endogenous C-region is a human C-region, then thereby humanisierte anti-bodies can be manufactured.

In a further preferential execution form of the integration vector according to invention the selectable marker is gpt, neo or coded for Hygromycin resistance. The specialist is with breeding of cells under selection conditions, which require these markers, trusts (see. e.g. Sambrook et al., a.a.O.). It is beyond that able to select further selection markers which can be used in the vector according to invention.

In a particularly preferred execution form the integration vector according to invention carries the designation pSV232Agpt-hu gamma 1-X5. Its construction is described in example 1.

Furthermore the invention concerns a procedure for the production of rekombinanten anti-bodies, with which one accomplishes the following steps

(A) Transfektion of anti-body-producing cells with the vector according to invention;
(B) Selection of stable Transformanten; and
(C) Identification the desired anti-body producing cells.

The Transfektion of anti-body-producing cells is considered as standard technique of the modern Immunologie. The specialist it is well-known that the Transfektionsbedingungen for each used Zellinie must be adjusted. A manual for the establishment of such Transfektionsbedingungen is for example in Toneguzzo et al., mol. Cell Biol. 6 (1986), 703-706 as well as in the description to the bio wheel ?gene pulsating machine? given. For example for the Mausmyelomlinie NS-1 (ATCC TIB 18) suitable Transfektionsbedingungen is described in Mocikat et al., genes 136 (1993), 349-353. The selection of stable Transformanten takes place via breeding the Transformanten for at least 7 days in a suitable selection medium. The selection of stable Transformanten is necessary, in order to kill cells, which did not take up the Plasmid. The choice of the selection medium depends naturally on the used selection marker. The production of suitable selection media is while stationary the technology well-known and for example in Sambrook et al., a.a.O. after-readably.

The identification the desired anti-body-producing cells can take place for example through for the constant region specific anti-bodies, if the DNA sequence in the integration vector codes a C-gene or a domain of it. However if a V-gene is in-recombined by the integration vector into a functionally rearrangiertes endogenous anti-body gene, then the Expression of the desired anti-body can be determined by anti- idiotypische anti-bodies. As test procedures for example RIAs or ELISAs are suitable. Also these procedures belong to the standard repertoire of the average specialist. Beyond that the integration of the transferred domain (n) with that knows while stationary the technology admitted PCR procedures to be determined. The specialist is able to determine for this suitable primers and reaction conditions.

In a preferential execution form of the procedure according to invention the Transfektion takes place via Elektroporation, calcium Kopräzipitation, Lipofektion, the DEAE Dextran technology or retroviralen gene transfer. All these procedures are while stationary the technology well well-known. The specialist knows how he has to stop the conditions for the individual Transfektionsverfahren in the procedure according to invention.

In another preferential execution form of the procedure according to invention takes place the selection in a medium, which contains Mykophenolsäure, G418, or Hygromycin as selection means. As mentions already managing, these selection means are while stationary the technology well well-known. Their choice depends on the used selection marker, while their dosage from standard works of molecular biology can be deduced; see. e.g. Sambrook et al., a.a.O.

In another preferential execution form of the procedure according to invention the DNA sequence codes constant domains gamma of the 1, gamma 2a, gamma 2b, gamma 3, gamma 4, mu -, alpha -, delta - or epsilon - of type of ISO.

In a further preferential execution form of the procedure according to invention the additionally following step is accomplished:

(there) Retransfektion of the anti-body-producing cells with the integration vector according to invention, which used another selection marker than in step (A) exhibits; or
(railways) Retransfektion of the anti-body-producing cells with an integration vector, which contains at least a functional Enhancer and which another selection marker than in step (A) used exhibits.

The functional Enhancer is preferably the C mu -, C kappa - or SV40-Enhancer.

The Zellinien produced by homologous recombination exprimieren often additionally to the desired rekombinanten anti-bodies further the original endogenous anti-body. This is on the presence of several copies of functionally rearrangierter alleles in the anti-bodies producing cell to lead back for example a Hybridomzelle. With the procedure according to invention a such unwanted Doppelproduktion is switched off by anti-bodies. A stable Transformante received after the selection from step (B), which sezerniert after homologous recombination two anti-body species, will according to invention with the same integration vector, which is provided with another selection marker gene however, as to the first Transfektion of step (A) used or with an appropriate Enhancer containing integration vector, was retransfiziert. A such exchange of genes for selection markers knows simple DNA Rekombinationstechniken after while stationary the technology admitted to be achieved. Possible exchangeable selection marker genes were mentioned managing in this description. After selection on the second selection marker clone are established, which only the desired rekombinanten anti-body exprimieren. A further increase of the anti-body production rate is reached by this second Transfektion and following selection beyond that.

Finally the invention concerns kits for the production of rekombinanten anti-bodies, which contain at least a vector according to invention. These kits can be used for the easy production of anti-bodies with desired specificities or desired ISO types. They offer a means to the specialist to isolate with relatively little expenditure wished rekombinante anti-body. The kits according to invention contain either one or more vectors according to invention. They can contain further components beyond that, as for example suitable selection means.

The figures show:

Figure 1: schematic representation of an execution form of the integration vector according to invention, with which in a functionally rearrangiertes mouse gamma 2a-Gen the C-Exons for the human gamma 1-Gen is inserted. The vector is linearized before the Transfektion into a suitable mouse Hybridomzellinie with the restriction enzyme XbaI. After the Transfektion integrates the vector in the place marked by X. Parts of the mouse gene COM are duplicated by the integration. In such a way received recombination product is likewise schematically represented.
Figure 2: Schematic representation of the construction of the vector pSV232Agpt-hu gamma 1-X5. The individual construction steps are more exactly in example 1 described.

The examples describe the invention.

Example 1: Vector construction

The human constant IgG1-Gensegmente becomes as 2.9 kbEcoRI PvuII fragment (Ellison et al., Nucleic Acids Res. 10 (1982), 4071-4079) into the vector split with EcoRI and BamHI psVgpt (Mulligan and mountain, Proc. Natl. Acad. Sci. The USA 78 (1981), 2072-2076), its BamHI place kloniert by treatment with Klenow enzyme (2 units, 30 min. with 37 DEG C) provided with smooth ends is. Into the EcoRI place upstream from the C-Exons becomes afterwards over smooth ends (after Klenow treatment, 2 units, 30 min. with 37 DEG C) 2.3 a KB-HindIII-fragment inserted, which contains a part of the mouse mu - Introns including mu - of Enhancers as well as the JH4-Segment (Gough and Berne pool of broadcasting corporations, Proc. Natl. Acad. Sci. The USA 78 (1981), 509-513; Banerji et al., Cell 33 (1983), 729-740). From this Konstrukt with PvuI and HindIII a range is cut out, the parts of the pBR vector, the murine flank, which contains human IgG1-C-Exons as well as parts of the gpt Expressionseinheit without the SV40-Enhancer. This fragment becomes with 3.7 KB a large range from the Plasmid pSV232Agpt (Kadesch and mountain, mol. Cell. Biol. 6 (1986), 2593-2601) ligiert, which was isolated by splitting with PvuI and HindIII from this vector. This fragment does not contain the SV40-Promotor, as well as one any longer functional part of the SV40-Enhancers. Finally the 0.7 KB long sequence is eliminated downstream by the EcoRI place by splitting with EcoRI and SacI from the vector and by 1.4 a KB fragment from the murinen mu - Intron replaces, so that the entire Homologieflanke exhibits a length of 3,0 KB. From this flank it becomes by splitting with XbaI whereby a fragment of 1,0 KB develops, and the range mu - of the Intron Enhancers removes Rezirkularisierung. The resulting vector is marked with pSV232Agpt-hu gamma 1-X5; see. Figure 2.

Example 2: Transfektionsexperiment

The Transfektion 10< becomes; 7> exponentially growing Hybridomzellen abzentrifugiert and in 700 mu l ice-cold RPMI 1640 (Gibco, BRL) resuspendiert. 20 mu g pSV232Agpt-hu gamma 1-X5 are präzipitiert with XbaI completely linearized, with isopropanol, bidest in 20 mu l H2O. resuspendiert and to the cell suspension given. The Transfektion takes place by means of Elektroporation (bio wheel, type ?gene pulsating machine?) with an individual current pulse; Field strength and condenser capacity are individually stopped for each Zellinie. The specialist is with the attitude of these conditions trusts, those for example in the operating derivative to the ?gene pulsating machine? or in Toneguzzo et al., a.a.O. are in detail shown. The cells are held 10 minutes ice and then in a density of 10< 5> Cells per cavity on 24-Loch-Platten in RPMI 1640, supplementiert with 10% foetalem calf serum (Gibco, BRL) and 2mM Glutamin (Gibco, BRL) distributes.

Example 3: Selection of stable Transfektanten

After 48 hours the Transfektion begins the selection with rising concentrations from Mykophenolsäure to a final concentration of 2 mu g/ml in presence of 250 mu g/ml Xanthin and 15 mu g/ml Hypoxanthin. The clones are searched for stable growth in the distance of 2 days. The projections of stably growing clones are searched for their content of human IgG1. In addition a ELISA with Ziegeanti human IgGFcspecific catch he anti-body and peroxidase-coupled detection anti-body the same specificity is used (see. Example 4). Clones, whose projection supplies a positive result, are expanded.

Example 4: Enzyme-bound absorption test

ELISA-Platten (Nunc) werden mit Ziege-anti-human-IgGFc-spezifischem Fänger-Antikörper (Dianova, Hamburg) beschichtet. Free connection places are blocked with 1% milk powder. Subsequently, the culture projections which can be tested are inkubiert and the test afterwards with Peroxydase coupled detection anti-body (dia. new facts) is inkubiert. After everyone of the managing steps the plates are extensively washed with phosphate-buffered of physiological saline solution. The test is developed with o-Phenylendiamin (sigma). Everyone that reactions managing specified is accomplished one hour at ambient temperature. The color reaction is measured in a ELISA measuring instrument (STLLabinstruments, type SF+) with a wavelength by 405nm.

After the Transfektion of 10< 7> Hybridomzellen will receive 20 Mykophenol resistant clones. After statistic evaluation of it 5% the human gamma 1-Isotyp exprimieren. The integrity of the human heavy chain became in the SDSPAGE gel and in the Western Blot (see. Example 5) confirms.

Antigen connection specificity and the affinity correspond to that of the parentalen mouse anti-body, as by Kompetitionsversuche (see. Example 6 was proven).

Example 5: Western Blot

The rekombinanten anti-bodies are cleaned from the culture projection over a FPLC protein g Sepharose column (Pharmacia, Freiburg). With 2 mu g protein is accomplished a SDS PAGE (gradient of 8-15% acrylamide) after standard techniques. Die Proteine werden durch Elektroblotten auf eine Nitrocellulosemembran übertragen und durch Ziege-anti-human-IgGFc-Antikörper, der an Meerrettichperoxidase gekoppelt ist (Dianova) nachgewiesen. The color development is introduced by addition by 3,3-Diaminobenzidin.

Example 6: Kompetitionsexperimente

Mouse Lymphozytenzellen, which carry the goal antigen, become 30 min. after standard techniques with dilution rows of the rekombinanten anti-body and afterwards 10 min. with the parentalen anti-body, which is marked with FITC, with 0 DEG C inkubiert. The loading of the cells with the marked anti-body is measured after standard techniques in the FACS.

Example 7: Elimination of the original endogenous rodent anti-body in a double producer resulted from homologous recombination

4.4 KB a large fragment, that from the Plasmid pSVneo (Southern and mountain, J. Mol. Appl. Genet. 1 (1982) is isolated 327-341) after splitting the Plasmids with PvuI and BamHI and that the neo-Gen carries, into the integration vector is built, which the functional SV40 and C mu - Enhancer contains and from with the same splitting the gpt gene was removed. Into this vector the SV40-Enhancer is replaced by the trunkierten SV40-Enhancer from pSV232Agpt and in addition mu - Intron Enhancer deletiert, as in example 1 and/or. Figure 2 described. A Zellinie, which sezerniert both an human and the parentalen type of mouse ISO, is transfiziert either with that Enhancer carrying or Enhancer loose, neo basic vector, as described 2 in example. Later the selection begins 48 hours with 1,5 mg/ml G418 (Gibco BRL), whereby the selection with Mykophenolsäure is maintained (see. Example 3). The projections of stable Transfektanten are searched in a ELISA, as in example described 4, whereby however as a fänger and as detection anti-body goat anti- mouse IgGFc is used. The clone, which do not exprimieren the murinen anti-body any longer, are expanded.

Usually the anti-body production rate with the procedure described in this example is improved around at least one factor 2 compared with the procedures described in the previous examples.

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1. Integration vector for the production of genes, which code rekombinante anti-body, which contains the following elements:

(A) a range from at least 1.5 KB, which homologous to a range mu - is Introns or k-Introns, which no or no functional C mu - or CC-Enhancerium contains;
(B) at least one DNA sequence, which codes a domain of an anti-body or a part of it; and
(C) a marker selectable in eukaryontischen anti-body-producing cells, which does not exhibit a functional Enhancer range, whereby the Expression of this marker is steered after the integration by cellular C mu - or CC-Enhancer.

2. Vector according to requirement 1, whereby the range mu - or k-Introns C mu - or CC-Enhancerlokus covers and the range from at least 1.5 KB no functional C mu - or CC-Enhancer contains.

3.Vektor according to requirement 1 or 2, whereby the DNA sequence codes a V-domain or a part of it.

4. Vector according to requirement 1 or 2, whereby the DNA sequence codes a constant region or a part of it.

5. Vector after one of the requirements 1 to 4, whereby the range, which is homologous to C mu - or CC-Enhancer comprehensive range mu - or k-Introns, at least 1.9 KB covers.

6. Vector after one of the requirements 1 to 4, whereby the range, which is homologous to C mu - or CC-Enhancer comprehensive range mu - or k-Introns, at least 2.0 KB covers.

7. Vector after one of the requirements 1 to 6, which contains a bacteriacompatible regularization unit.

8. Vector after one of the requirements 1 to 7, whereby the rekombinante anti-body is a chimärer anti-body.

9.Vektor after one of the requirements 1 to 8, whereby the DNA sequence codes domains of a human anti-body chain.

10. Vector after one of the requirements 1 to 8, whereby the DNA sequence codes domains, which originate from the mouse, rat, goat, from the horse or sheep.

11. Vector after one of the requirements 1, 2 and 4 to 10, whereby the DNA sequence codes all C-domains of a sekretorischen anti-body.

12. Vector after one of the requirements 1, 2 and 4 to 10, whereby the DNA sequence codes all C-domains of a diaphragm-bound anti-body.

13. Vector after one of the requirements 1, 2 and 4 to 12, whereby the Exons codes C-domains of an anti-body, which is a IgM, a IgG1, a IgG2a, a IgG2b, a IgG3, a IgG4, a IgA, IgD or IgE Antikörper.

14.Vektor after one of the requirements 1 to 13, whereby the rekombinante anti-bodies are producing cells human cells or mouse cells or hybrids of it.

15. Vector after one of the requirements 1 to 14, whereby the selectable marker gpt, neo, or a HygromycinResistenz of coding markers is.

▲ top 16. Vector according to requirement 15, which carries the designation pSV232Agpt-hu gamma 1-X5 and whose construction is described in example 1.

17. Procedure for the production of rekombinanten anti-bodies, with which one accomplishes the following steps:

(A) Transfektion of anti-body-producing cells with a vector after one of the requirements 1 to 16;
(B) Selection of stable Transformanten; and
(C) Identification the desired anti-body producing cells.

18.Verfahren according to requirement 17, whereby the Transfektion by Elektroporation, Calciumphosphat Kopräzipitation, Lipofektion, which takes place DEAE Dextran technology, or retroviralen gene transfer.

19. Procedure according to requirement 17 or 18, whereby the selection takes place in a medium, which contains Mykophenolsäure, G418 or Hygromycin as selection means.

20. Procedure after one of the requirements 17 to 19, whereby the Exons codes constant domains mu -, gamma 1, gamma 2a, gamma 2b, gamma 3, gamma 4, delta -, alpha - or epsilon - of type of ISO.

21. Procedure after one of the requirements 17 to 20, with which one accomplishes the additionally following step:

(there) Retransfektion of the anti-body-producing cells with the vector after one of the requirements 1 to 16, which used another selection marker than in step (A) exhibits; or
(railways) Retransfektion of the anti-body-producing cells with an integration vector, which contains at least a functional Enhancer and which another selection marker than in step (A) used exhibits.

22. Kit for the production of rekombinanten anti-bodies, containing at least one vector after one of the requirements 1 to 16.